Solid Phase Extraction (SPE) of Alkaline Drugs from Biological Fluids

1 Introduction

This procedure detects common alkaline drugs. It is derived from "Fentanyl and Analogues in Urine for GC or GC/MS Confirmations" published in the *Solid Phase Extraction Applications Manual* by United Chemical Technologies, Inc. The published procedure for extraction of biofluids is essentially followed intact to prepare a crude basic drug isolate.

2 Scope

This procedure allows for screening and confirmation of a wide variety of alkaline drugs in biological fluids. This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

Biological specimens are qualitatively assayed for basic drugs. Specimens are mixed with internal standards, adjusted to a slightly acidic pH, and extracted using Clean Screen DAU solid phase extraction cartridges. Basic drugs are eluted using a mixed solvent system of methylene chloride, isopropanol, and ammonium hydroxide. The eluent is taken to dryness and reconstituted prior to analysis by GC/MS(EI) (gas chromatography/mass spectrometry)(electron ionization) and/or LC/MS(ESI) (liquid chromatography/mass spectrometry)(electrospray ionization).

4 Specimens

This procedure uses a biological fluid such as: blood, serum, plasma, urine, vitreous humor, or a prepared tissue homogenate (1:1). When available, 1 mL of blood, other fluid, or tissue homogenate is used. This procedure may also be used to screen food samples for alkaline drugs, providing that appropriate controls are simultaneously analyzed. A 1 g sample of a food homogenate (1:1) is suggested for analysis.

5 Equipment/Materials/Reagents

a. Gas Chromatograph/Mass Spectrometer equipped with a 30 m x 0.25 mm x 0.25 µm Rtx-5MS (or equivalent) column.

b. Centrifuge

c. Evaporator w/ Nitrogen

d. Vortex mixer

e. SPE Vacuum or Positive Pressure Manifold

f. Desiccator

g. 16 x 100 mm screw-top tubes with Teflon insert caps

h. 13 x 100 mm culture tubes with polypropylene snap-tops

i. 12 x 75 mm culture tubes with polypropylene snap-tops

j. Clean Screen DAU® SPE cartridges (regular flow) - 200 milligrams

k. Water (18mΩ, HPLC, Optima, or UPLC grade)

l. Methanol (GC^2 grade, HPLC, Optima grade, or better)

m. 100 mM Phosphate Buffer (pH 6.0):
To a 500-mL volumetric flask, add 400 mL deionized water, 6.1 g sodium phosphate monobasic monohydrate, and 1.6 g sodium phosphate dibasic heptahydrate. Mix well to dissolve. Verify 5.8<pH<6.1. Bring to volume with deionized water. Store refrigerated in glass. Stable 2 months.

n. Elution Solvent (Methylene Chloride/Isopropanol/Ammonium Hydroxide (78/20/2)):
Combine 20 mL HPLC grade isopropanol with 2 mL ACS grade concentrated ammonium hydroxide and mix well. Add 78 mL HPLC or pesticide grade methylene chloride and mix well. Store in glass at room temperature. Prepare fresh daily.

o. 100 mM Acetic Acid:
To a 100-mL graduated cylinder, add 80 mL deionized water and 0.5 mL glacial acetic acid. Mix well and bring to 85 mL with deionized water. Store in glass at room temperature. Stable 6 months.

p. Mid-range pH paper

q. PTFE (0.5 micron) membrane

r. HPLC Column (Xterra C-18 MS, 3.0 x 150 mm, 3.5 µm dp; or equivalent)

s. Mobile Phase 1 (Water with 0.1% Formic Acid): Combine 500 mL Optima grade water and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable 2 weeks.

t. Mobile Phase 2 (Acetonitrile with 0.1% Formic Acid): Combine 500 mL Optima grade acetonitrile and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable for 1 month.

6 Standards and Controls

a. Alkaline Screen Internal Standard Working Solution:
Prepared by adding the following 100 µg/mL solutions (purchased from Cerilliant, or another approved supplier) to a 25-mL volumetric flask and diluting to the mark with methanol:

Analyte	Volume (mL)	Resulting concentration when 50 µL is added to 1.0 mL matrix (ng/mL)
d_5-fentanyl	1.0	200
d_3-benzoylecgonine	0.25	50
d_5-methamphetamine	0.125	25
d_3-morphine	0.125	25
d_3-oxycodone	0.125	25
d_3-ecgonine methyl ester	0.125	25
d_5-alprazolam	0.100	20

b. Negative Control:
Purchased from Cliniqa, Dynatek or an equivalent approved supplier, or prepared in-house from an appropriate blank specimen. Store refrigerated or obtain fresh. Stability determined by manufacturer. A Negative Control will be extracted and analyzed with every assay. When possible, the Negative Control will be matrix matched.

c. Alkaline Screen Control Working Solution (5 µg/mL):
Prepared by adding 0.25 mL of each of the following 1.0 mg/mL standards (purchased from Cerilliant, or another equivalent supplier) to a 50-mL volumetric flask and bringing to the mark with HPLC grade methanol:
Amitriptyline, amphetamine, cocaine, diphenhydramine, hydrocodone, nordiazepam, tramadol and venlafaxine.
This mixture is stored refrigerated in glass. Stable for at least one year.

d. Positive Control (200 ng/mL each anayte):
Prepared fresh by adding 0.040 mL of the Alkaline Control Working Solution (5 µg/mL) to 1 mL of Negative Control. Alternatively, other alkaline drugs and/or metabolites may be added to an aliquot of Negative Control to prepare controls for specific needs. A

Positive Control will be extracted and analyzed with every assay. When possible, the Positive Control will be matrix matched.

Note: When drugs are indicated in an alkaline drug screen that are not present in the Positive Control, a sample of the reference material (typically ~100 µg/mL for GC/MS or ~1-5 µg/mL for LC/MS) may be analyzed instrumentally without extraction to verify the retention time and spectra of the analyte in the unknown sample. For confirmatory assays, matrix matched positive controls that are extracted alongside the unknown sample when available.

e. Benzodiazepine Multi-Component Mixture-8 Stock Standard (250 µg/mL of alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, temazepam): Purchased from Cerilliant Corporation. Storage conditions and stability determined by manufacturer.

f. Benzodiazepine Multi-Component Mixture-8 Stock Standard Working Solution (5 ug/mL of alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, temazepam):
To a 50 mL volumetric flask add 1.0 mL of Benzodiazepine Multi-Component Mixture-8 Stock Standard and dilute to the mark with acetonitrile. Store <0°C in glass or plastic. Stable for at least 2 years.

g. Amine Mixture-6 Stock Standard (250 µg/mL of amphetamine, methamphetamine, phentermine, methylenedioxyamphetamine (MDA), methylenedioxymethyamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA)):
Purchased from Cerilliant Corporation. Storage conditions and stability determined by manufacturer.

h. Amine Mixture-6 Stock Standard Working Solution (5 µg/mL of amphetamine, methamphetamine, phentermine, MDA, MDMA, and MDEA):
To a 50 mL volumetric flask add 1.0 mL of Amine Mixture-6 Stock Standard and dilute to the mark with methanol. Store <0°C in glass or plastic. Stable for at least 2 years.

i. LC/MS Performance Standard (0.83µg/mL of alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, temazepam, amphetamine, methamphetamine, phentermine, MDA, MDMA, and MDEA):
Combine 0.025mL of the Benzodiazepine Multi-Component Mixture-8 Stock Standard Working Solution, 0.025mL of the Amine Mixture-6 Stock Standard Working Solution and 0.100mL of water. Prepare fresh or store mixture under refrigerated conditions. Stable for at least 2 weeks.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. To properly labeled 16 x 100 mm screw-top tubes add 1 mL of biological fluid or 1 g of prepared tissue homogenate (1:1 in deionized water). Also prepare Negative and Positive Controls for each matrix being analyzed.

b. Add 50 µL of the Alkaline Screen Internal Standard Working Solution to biological fluid specimens.[1] For tissue specimens add 100 µL of each Internal Standard Working Solution.

c. Add 4 mL of 100 mM phosphate buffer. Vortex. Verify pH is 6.0 ± 0.5.

d. For blood and tissue specimens: Centrifuge at high speed for 15 minutes. Transfer supernatant to a clean 13 x 100 mm culture tube, leaving solid cellular material behind. Bring volume up to 5 mL with deionized water. Verify pH is 6.0 ± 0.5.

e. Pre-rinse SPE extraction cartridge by adding 3 mL of methanol at 1 mL/minute.

f. Condition cartridge with 3 mL of deionized water followed by 1 mL of 100 mM phosphate buffer at 1 mL/minute. Do not allow sorbent to dry.

g. Load sample on SPE cartridge at 1-2 mL/minute. Do not allow sorbent to dry.

h. Wash cartridge with 3 mL of deionized water, 1 mL of 100 mM acetic acid, and 3 mL of methanol (each at 1-2 mL/minute).

i. Dry cartridge under full vacuum for 3 minutes.

j. Apply 3 mL of Elution Solvent at 1-2 mL/minute. Collect eluent in 12 x 75 mm test tubes.

k. Evaporate to dryness under nitrogen at 40°C.

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[1]Other internal standards may be substituted at relevant concentrations if deemed appropriate.

l. Analyze the extracts using one or both of the instrumental techniques that follow.

1. For GC/MS(EI) analysis, reconstitute the dry residues with 40-50 µL of GC[2] grade methanol and analyze 2 µL following the instrumental parameters given below after confirming that the instrument is in proper working condition.

2. For LC/MS(ESI) analysis, add 100 µL water to the methanol extract prepared above and analyze 10 µL following the instrumental parameters given below after confirming that the instrument is in proper working condition.

9 Instrumental Conditions[2,3]

9.1 GC-MS (EI)

9.1.1 Gas Chromatograph Parameters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	60°C	inlet temperature	220°C	type	HP5-MS
hold 1	2 min	injection mode	split	length	30 m
ramp 1	35°C/min	carrier gas	ultrapure helium	internal diameter	0.25 mm
temperature 2	280°C	carrier mode	constant flow	film thickness	0.25 µm
hold 2	26.71 min	carrier flow	1.2 mL/min		
total run time	35 min	split flow	12 mL/min		
		split ratio	10:1		
		Injection volume	2 µL		

[2] Instrumental conditions may be modified to account for particular target analytes. Any modifications will be recorded in case notes.
[3] Appendix 2 contains an abbreviated version of instrumental parameters used in this procedure. This checklist may be used by the examiner or chemist performing the procedure.

9.1.2 Mass Spectrometer Parameters

ionization mode	electron impact (+)	Source/Quad temperatures	230/150°C
scan mode	full scan	transfer line temperature	280°C
scan range	35 – 500 AMU	solvent delay (nominal)	5 min*

*This value may be shortened as a column ages and is clipped to ensure that amphetamine elutes after the solvent delay.

9.2 LC-MS(ESI)

Mobile Phase Composition	Flow Parameters			Column Parameters	
MP1: Water with 0.1% Formic Acid	total flow		0.3 mL/min	type	Xterra C-18 MS
	time (min)	%MP1	%MP2	length	150 mm
MP2: Acetonitrile with 0.1% Formic Acid	0	90%	10%	internal diameter	3 mm
	5	90%	10%	particle size	3.5 µm
	20	10%	90%	temperature	30°C
	31	90%	10%		
	37	90%	10%		
	total run time		37 min		
Autosampler:	Injection Volume		10 µL	Temperature	15°C

9.2.1 Mass Spectrometer Parameters[4]

Source	Electrospray (ESI, +)	Segments	5	Events per segment	7
Segment times	#1: 0-12 min; #2: 12-13.5 min; #3: 13.5-14 min; #4: 14-15 min; #5: 15 min - end				

Events #1 and 5	
Mode	full scan MS
Range	100 – 650 m/z
Resolution	FTMS; 15000

Events #2-4		
Full Scan MS/MS	Data Dependent Product Ion	ITMS – unit resolution

[4]Different MS/MS parameters (i.e., higher collision energy or MS^3) may be used to target specific drugs and metabolites as long as the same parameters are used for all controls and case samples and the method is recorded in the case notes.

MS/MS of 1st, 2nd and 3rd most intense ions in Event #1 from segment specific target lists (See Appendix 3 and 4)

Events #6-7		
Full Scan MS/MS	Data Dependent Product Ion	ITMS – unit resolution
MSMS of 1st and 2nd most intense ions in Event #5		

Note: For target analysis, method may be reduced to fewer time segments. Additionally, Scan Events 5-7 may be removed and target ion(s) can be added to or removed from Events 2-4.

10 Decision Criteria

10.1 LC/MS Performance Standard Decision Criteria

In addition to the performance checks specified in the LC/MS standard operating procedure, a performance standard mix is analyzed through the analytical column to monitor the performance of the column.

10.1.1 Chromatography

In order for the LC to be considered in good operating condition, molecular ion traces for each analyte in the performance standard should have reasonable peak shape. Over time, the amines in the performance standard mix may demonstrate wider chromatographic peaks, indicating deterioration of the packing of the analytical column.

The retention times of the 14 analytes from the performance standard should be within ±0.5 min of the previous run (same instrument and same column) for benzodiazepines and within ±0.6 min for sympathomimetic amines . Minor changes in mobile phase percentage may account for slight retention time shifts. Over time, the amines in the performance standard mix may give longer and longer retention times, indicating deterioration of the packing of the analytical column or a problem with the frit. When the amine peak shape becomes poor or retention times are >0.6 min longer than the last run, the frit should be replaced. Replacement of the column is also a consideration.

The areas of each chromatographic molecular ion peak in the performance standard should be comparable (within 50% - 200%) to the previous run of the performance standard.

10.1.2 Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the 14 analytes in the performance standard will be present. (The following molecular

ions (high resolution ion listed in parentheses) will be present as the base peak for each analyte: clonazepam – 316 (316.048), nitrazepam – 282 (282.087), flunitrazepam – 314 (314.094), lorazepam – 321 (321.019), oxazepam – 287 (287.058), alprazolam – 309 (309.090), temazepam – 301 (301.074), diazepam – 285 (285.079), amphetamine – 136 (136.112), methamphetamine and phentermine – 150 (150.128), MDA – 180 (180.102), MDMA – 194 (194.118), and MDEA – 208 (208.133).)

10.2 Unknown Sample Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay.

10.2.1 Batch Acceptance

No analytes of interest will be detected in the Negative Control. For this purpose, analytes of interest are defined as any analytes that are being reported for this batch.

Each of the eight analytes in the Positive Control will be detected in either the GC/MS data, the LC/MS data, or both.

10.2.2 Unknown Sample Acceptance

The d5-fentanyl will be detectable in the GC/MS data.

The d5-fentanyl, d5-alprazolam, d5-methamphetamine, d3-morphine, d3-oxycodone, d3-benzoylecgonine and d3-ecgonine methyl ester will be detectable in the LC/MS data.

10.2.3 Unknown Sample Compound Identification

In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard or extracted Positive Control.

In situations where a reference material is not available for comparison, it may still be possible to identify a suspected compound in an extract[5]. In such circumstances, chromatographic fidelity and signal-to-noise requirements must be met, and the mass spectrum for the analyte of interest must compare favorably to a published reference library spectrum. Also, there must be strong additional supporting information that reasonably suggests the presence of the suspected compound. For example, a drug metabolite for which no reference standard is available might be considered identified on the basis of a good quality mass spectral library match combined with identification of the parent drug in the same sample through comparison to a reference standard.

[5]See *General Approach to Report Writing for Toxicology* for details on how to report a positive finding when no reference standard is available.

10.2.3.1 Chromatography

The peak of interest will show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample will compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.2.3.1.1 GC Retention Time

The retention time of the peak will be within ±2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, an extracted Positive Control or an appropriate deuterated analog.

10.2.3.1.2 LC Retention Time

The retention time of the peak will be within 5% or ±0.5 min (whichever is greater) of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, an extracted Positive Control, or an appropriate deuterated analog.

10.2.3.1.3 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio will exceed 3. Further, the baseline signal for the peak of interest will be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

10.2.3.2 Mass Spectrometry

The mass spectrum of the analyte of interest will match that of a reference standard, extracted calibrator, or an extracted Positive Control. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for further guidance.

10.3 Reporting EDDP

Methadone is known to break down to EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, a metabolite of methadone) in solution. To avoid false positive identification of EDDP in a sample, the following criteria must be met to report EDDP using this method: The ratio of the EDDP area (sum of the m/z 276 and m/z 277 ions in GC/MS data) to the methadone area (m/z 72 ion in GC/MS data) must be > 5% to report EDDP in a sample.

10.4 Reporting Cocaine

To report cocaine qualitatively based upon this method, the area of the M+H peak for cocaine (from the LC/MS data) must be greater than or equal to 5% of the M+H peak for benzoylecgonine (from the same sample).

10.5 Screening LC/MS Data

To screen high resolution LC/MS data for a wide number of analytes, M+1 ions for dozens of analytes may be traced at a mass tolerance of ±5 mmu.

10.6 Cautionary Note

Diphenhydramine, doxylamine, norsertraline and other drugs may fragment in the ESI source.

10.7 Planned Action on QC Failure

Refer to Quality Control for Toxicology Examinations (TOX101) for guidance on action steps in the event of a quality control failure.

10.8 Reporting Cut-offs for College of American Pathologists (CAP) T Series and FTC Series:

See Quality Control for Toxicology Examinations (TOX101) for guidance on estimating the amount of an analyte in a specimen. When analyzing CAP T-Series or FTC specimens, if all decision criteria for an analyte of interest are met, but the concentration of 6-acetylmorphine, buprenorphine, fentanyl, norfentanyl, and/or norbuprenorphine is estimated to be below 5 ng/mL (or 15 ng/mL for all other alkaline drugs) in two independent analyses, the analyte will not be reported. Note: the second analysis may be a repeat of this procedure or via another validated procedure. A Positive Control at the Cut-off Level is recommended for the second analysis.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limit of Detection: While the limit of detection varies depending upon analyte and matrix, this assay readily detects a wide variety of alkaline drugs and metabolites in blood and urine specimens at concentrations of 5 ng/mL. See Appendix 3 and 4.

b. Interferences: None known. Grossly decomposed or putrefied samples, as well as samples that have been embalmed, may affect detection limits.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

"Fentanyl and Analogues in Urine for GC or GC/MS Confirmations", *Solid Phase Extraction Applications Manual*, United Chemical Technologies, 2005.

Baselt, R.C. and Cravey, R.H. *J Anal Tox.* 1977**,** *1*, 81-103.

Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.

Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

Winek, C. *Drug and Chemical Blood-Level Data*, 1994.

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

Quality Control for Toxicology Examinations (TOX101); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

General Approach to Report Writing for Toxicology (Tox 106); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

ELISA Screening (Tox 209); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
8	06/18/18	Section 2: updated scope language. Removed "subunit" from header and multiple locations. Section 3: defined EI and ESI. In Section 5-k specified several types of water that can be used (also removed DI from last location in procedure bench sheet). In section 5-l: added "Optima grade or better" (also removed 'HPLC' from procedure bench sheet). Corrected typos in Section 5 (s, t). Section 6-d: changed to "when available". Section 8 (e, h): removed "HPLC". Section 8-l: removed sentence describing combined GC/MS and LC/MS workflow, since LC/MS only workflow now also an option. Updated Section 8-l.2. Section 9.2: added LC autosampler parameters. Section 9.1.2: added quadrupole temperature of 150°C, updated instrument bench sheet and added "nominal" to mass spec solvent delay time. Throughout section 10: changed multipole instances of "should" to "will". Section 10.1.1; clarified 14 analyte source. Section 10.3: defined EDDP. Removed "electron impact" from Section 10.2.3. Added Section 10.7 which references the updated TOX101 QC guidance document concerning planning action on QC failure. Added TOX101 to References. Renamed references to TOX106. Section 13: added reference to Appendix 3. In Appendix 3, updated dextromethorphan accurate mass from 272.200 to 272.201 m/z.
9	11/15/19	Revised 6i concentration and expiration date. Updated grammar 8l. Revised 9.2 mobile phase gradient hold and total run time and clarified 9.2.1 note section. Revised 10.1.1 and 10.2.3.1.2 retention time criteria. Added 10.8 reporting cut-offs for CAP proficiency tests. Revised Appendix 2 mobile phase gradient hold time. Appendix 3 - removed duplicate entries for olanzapine and phenazepam, updated LOD values for gabapentin, olanzapine, phenazepam, and ziprasidone.

Approval

Redacted - Signatures on File

Acting Toxicology Technical Lead: Date: 11/14/2019

Chemistry Unit Chief: Date: 11/14/2019

Appendix 1: Abbreviated version of the Alkaline SPE Procedure for bench use.

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Appendix 2: Abbreviated version of the Instrumental Parameters for bench use.

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Appendix 3: List of Analytes, approximate retention times and M+1, alphabetized

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
25B-NBOME	380.086	14.95	5	5	OK
25C-NBOME	336.136	14.78	5	5	OK
25H-NBOME	302.175	14.10	5	5	OK
25I-NBF	416.052	14.92	5	5	OK
25I-NBMD	442.051	15.00	5	5	OK
25I-NBOH	414.056	14.61	5	5	OK
25I-NBOME	428.072	15.15	5	5	OK
3-methoxyPCP	274.217	13.80	5	5	OK
6-AM	328.154	7.66	5	5	BP
7-aminoclonazepam	286.074	11.19	5	5	OK
7-aminoflunitrazepam	284.119	12.51	5	5	OK
acetylfentanyl	323.212	13.14	5	5	OK
alfentanyl	417.261	13.60	5	5	OK
alpha-PVP	232.170	12.29	5	5	OK
alprazolam	309.090	16.55	5	5	OK
amitriptyline	278.190	14.95	5	5	OK
amlodipine	409.152	14.72	25	25	OK
amoxapine	314.105	14.08	5	5	OK
amphetamine	136.112	6.05	25	25	BP
aripiprazole	448.155	14.84	25	25	OK
asenapine	286.099	14.34	5	10	I, MI
atenolol	267.170	3.44	5	10	SP
atomoxetine	256.170	14.50	5	5	OK
atropine	290.175	11.16	5	5	OK
baclofen	214.063	8.04	NS	NS	I
benzocaine	166.086	15.59	5	I	I
benzoylecgonine	290.139	11.70	5	5	OK
benztropine	308.201	15.04	5	5	OK
brexpiprazole	434.190	14.32	5	10	OK
bromazepam	316.008	14.70	5	10	OK
brompheniramine	319.080	12.83	5	5	OK
bupivacaine	289.227	13.28	5	5	I
buprenorphine	468.311	14.14	5	5	OK
bupropion	240.115	12.98	5	5	OK

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
buspirone	386.255	13.23	5	5	OK
butylone	222.112	9.85	5	5	BP
butyrylfentanyl	351.243	14.27	5	5	OK
BZP (benzylpiperazine)	177.139	2.25	5	10	SP
carbinoxamine	291.126	12.62	5	5	OK
carfentanil	395.233	14.19	5	5	OK
cetirizine	389.163	15.26	10	5	I
chlordiazepoxide	300.090	13.02	5	5	OK
chlorpheniramine	275.131	12.52	5	5	OK
chlorpromazine	319.103	15.26	5	5	OK
citalopram	325.171	14.15	5	5	OK
clobazam	301.075	18.36	NS	NS	NS
clomipramine	315.162	15.45	5	5	OK
clonazepam	316.048	16.77	NS	NS	NS
clonidine	230.025	5.20	5	5	BP
clozapine	327.137	12.94	5	5	OK
cocaethylene	318.170	13.34	5	5	OK
cocaine	304.154	12.62	5	5	OK
codeine	300.159	5.10	5	5	BP
cotinine	177.102	2.28	5	I	I
cyclobenzaprine	276.175	14.77	5	5	OK
cyproheptadine	288.175	14.74	5	5	OK
desalkylflurazepam	289.054	17.10	5	5	OK
desipramine	267.186	14.64	5	5	OK
desloratadine	311.131	12.11	25	25	I
desmethylcitalopram	311.155	14.04	5	5	OK
desmethylclomipramine	301.147	15.32	5	5	OK
desmethylclozapine	313.121	12.44	10	10	OK
desmethylcyclobenzaprine	262.159	14.65	5	5	OK
desmethyldoxepin	266.154	14.10	5	5	OK
desmethylflunitrazepam	300.078	16.34	NS	NS	NS
desmethyltapentadol	208.170	12.20	5	5	OK
desmethyltrimipramine	281.201	14.97	5	5	OK
desmethylvenlafaxine	264.196	11.38	5	5	OK
dextromethorphan	272.201	13.70	5	5	OK
dextrorphan	258.185	11.84	5	5	OK

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
diazepam	285.079	18.14	5	5	OK
dihydrocodeine	302.175	4.67	5	5	OK
diltiazem	415.169	14.22	5	5	OK
diphenhydramine	256.170	14.00	5	5	OK
donepezil	380.222	13.61	5	5	OK
doxepin	280.170	14.21	5	5	OK
doxylamine	271.180	6.00	5	5	BP
duloxetine	298.126	14.84	5	10	OK
EDDP	278.190	14.46	5	5	OK
EEE	214.144	2.26	25	25	BP
EME	200.128	2.10	5	5	BP
estazolam	295.075	16.30	5	5	OK
etizolam	343.077	17.11	5	5	OK
fentanyl	337.227	13.80	5	5	OK
fexofenadine	502.295	14.87	5	5	OK
flubromazolam	371.030	16.58	5	5	OK
flunitrazepam	314.094	17.35	NS	NS	NS
fluoxetine	310.141	15.18	5	5	OK
fluphenazine	438.182	15.15	5	10	OK
flurazepam	388.159	13.94	5	5	OK
fluvoxamine	319.163	14.77	5	5	OK
furanylfentanyl	375.207	14.00	5	5	OK
gabapentin	172.133	5.29	Unk*	Unk*	*Unk
guaifenesin	199.096	12.75	NS	NS	NS
haloperidol	376.149	14.33	5	5	OK
hydrocodone	300.159	8.17	5	5	BP
hydromorphone	286.144	3.55	5	5	SP
hydroxyzine	375.183	14.77	5	5	OK
iloperidone	427.203	14.36	5	5	OK
imipramine	281.201	14.76	5	5	OK
ketamine	238.099	11.16	5	5	OK
lacosamide	251.139	12.79	NS	NS	NS
lamotrigine	256.015	11.89	5	5	OK
lidocaine	235.180	10.37	5	5	OK
loperamide	477.230	15.77	5	5	OK
loratadine	383.152	15.93	5	5	I

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
lorazepam	321.019	16.58	NS	NS	NS
lormetazepam	335.035	17.75	NS	NS	NS
loxapine	328.121	14.31	5	10	I
LSD	324.207	12.97	5	5	SP
lurasidone	493.263	15.62	5	5	OK
maprotyline	278.190	14.86	5	5	OK
MBDB	208.133	11.50	5	5	OK
MDA	180.102	7.13	5	5	BP
MDEA	208.133	10.72	5	5	OK
MDMA	194.118	8.44	5	5	BP
MDPV	276.159	12.57	5	5	OK
meclizine	391.194	16.63	5	5	OK
medazepam	271.100	13.88	5	5	OK
meperidine	248.165	12.69	5	5	OK
mephedrone	178.123	10.30	5	5	OK
mescaline	212.128	6.12	5	5	BP
mesoridazine	387.156	13.79	5	5	OK
metaxolone	222.112	16.81	NS	NS	NS
methadone	310.217	15.02	5	5	OK
methamphetamine	150.128	7.35	5	5	BP
methocarbamol	242.102	13.37	NS	NS	NS
methoxetamine	248.165	11.92	5	5	OK
methylone	208.099	5.63	5	5	BP
methylphenidate	234.149	12.10	5	5	OK
metoclopramide	300.147	11.60	5	5	OK
metoprolol	268.194	12.00	5	5	OK
midazolam	326.085	13.81	5	5	OK
mirtazapine	266.165	11.34	5	5	OK
molindone	277.191	11.95	NS	NS	NS
morphine	286.144	2.48	5	5	SP
MT45	349.264	14.90	5	5	BP
naloxone	328.154	4.89	5	5	BP
N-desmethyltramadol	250.180	12.08	5	5	OK
nicotine	163.123	2.06	5	I	I
nifedipine	347.124	18.13	NS	NS	NS
nitrazepam	282.087	16.26	5	5	OK

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
norbuprenorphine	414.264	12.99	5	5	OK
norchlorcyclizine	287.131	14.76	25	25	I
norchlordiazepoxide	286.074	12.88	5	5	OK
norcodeine	286.144	4.69	5	5	BP
nordiazepam	271.063	16.60	5	5	OK
norfentanyl	233.165	11.37	5	5	OK
norfluoxetine	296.126	15.02	5	5	OK
norhydrocodone	286.144	7.62	5	10	BP
norketamine	224.084	10.83	5	5	OK
normeperidine	234.149	12.64	5	5	OK
normorphine	272.128	2.46	25	25	I
noroxycodone	302.139	6.43	5	5	BP
norpheniramine	227.154	5.63	5	5	BP
norpropoxyphene	326.211	14.79	5	5	OK
norquetiapine	296.122	13.12	5	5	OK
norsertraline	292.065	15.10	25	25	OK
nortriptyline	264.175	14.83	5	5	OK
norverapamil	441.275	14.75	5	5	OK
O-desmethyltramadol	250.180	7.65	5	5	BP
OH-alprazolam	325.085	15.86	5	5	OK
OH-bupropion	256.110	12.00	5	5	OK
OH-midazolam	342.080	13.76	5	5	I
OH-quetiapine	400.169	8.62	10	10	BP
OH-risperidone	427.214	12.71	5	5	OK
OH-triazolam	359.046	15.90	NS	NS	NS
olanzapine	313.148	5.13	100	NS	BP
orphenadrine	270.185	14.50	5	5	OK
oxycodone	316.154	6.79	5	5	BP
oxymorphone	302.139	2.48	5	5	SP
paroxetine	330.150	14.58	5	5	OK
pentylone	236.128	12.00	5	5	OK
perphenazine	404.156	14.66	NS	NS	NS
phenazepam	348.972	17.81	100	NS	NS
phenethylamine	122.096	4.23	5	5	BP
pheniramine	241.170	6.52	5	5	BP
phentermine	150.128	9.10	5	5	BP

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	M+1	RT (min)	LOD	LOD	Notes
phenycyclidine	244.206	13.48	5	5	OK
phenylephrine	168.102	2.41	5	5	SP
phenylpropanolamine	152.107	3.83	25	25	OK
PMA	166.123	7.94	5	5	BP
PMMA	180.138	9.54	5	5	BP
prazepam	325.111	20.23	5	5	OK
pregabalin	160.133	5.25	NS	NS	NS
procainamide	236.176	2.48	5	5	SP
prochlorperazine	374.145	14.90	25	25	BP
promethazine	285.142	14.40	5	5	OK
propoxyphene	340.227	14.94	5	5	OK
propranolol	260.165	13.60	5	10	OK
protriptyline	264.175	14.67	5	5	OK
pseudo/ephedrine	166.123	4.70	5	5	BP
psilocin	205.134	4.45	NS	NS	NS
psilocybin	285.100	3.24	NS	NS	NS
quetiapine	384.174	13.48	5	5	OK
quinine/quinidine	325.191	9.46	I	I	I
ranitidine	315.149	3.66	10	10	SP
risperidone	411.219	12.68	5	5	OK
ropinirole	261.196	11.08	5	5	OK
scopolamine	304.154	6.88	5	5	BP
sertraline	306.081	15.24	5	5	OK
strychnine	335.175	10.84	5	5	OK
suvorexant	451.164	20.74	5	5	OK
tapentadol	222.185	12.31	5	5	OK
temazepam	301.074	17.40	NS	NS	NS
tetrahydrozoline	201.139	9.87	5	5	BP
tetrazepam	289.111	16.37	5	5	OK
TFMPP	231.110	13.15	5	5	OK
thioridazine	371.161	15.80	100	100	OK
tizanidine	254.026	4.51	5	5	OK
tramadol	264.196	11.99	5	5	OK
trazodone	372.159	13.30	5	5	OK
triazolam	343.051	16.79	5	5	OK
trifluprom azine	408.172	15.44	5	10	OK

Analytes Sorted Alphabetically			**Blood**	**Urine**	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
trimipramine	295.217	15.08	5	5	OK
triprolidine	279.186	12.91	5	5	BP
U47700	329.118	13.72	5	5	SP
venlafaxine	278.211	13.04	5	5	OK
verapamil	455.290	14.86	5	5	OK
W18	422.094	20.04	NS	NS	NS
zaleplon	306.135	16.08	NS	NS	NS
ziprasidone	413.120	13.82	25	100	OK
zolpidem	308.176	12.79	5	5	OK
zolpidem metabolite	338.150	11.19	5	5	OK
zopiclone	389.112	12.00	10	5	OK

Not suitable for analysis by this method	NS
LOD not thoroughly evaluated but consistently present in control spiked at 5 µg/mL	Unk*
Peak shape is broad; less than ideal	BP
Compound known to give split peaks	SP
Compound validated with no issues	OK
Matrix Interference	I

Appendix 4: List of Analytes, approximate retention times and M+1, in retention time order

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
nicotine	163.123	2.06	5	I	I
EME	200.128	2.10	5	5	BP
BZP (benzylpiperazine)	177.139	2.25	5	10	SP
EEE	214.144	2.26	25	25	BP
cotinine	177.102	2.28	5	I	I
phenylephrine	168.102	2.41	5	5	SP
normorphine	272.128	2.46	25	25	I
morphine	286.144	2.48	5	5	SP
oxymorphone	302.139	2.48	5	5	SP
procainamide	236.176	2.48	5	5	SP
psilocybin	285.100	3.24	NS	NS	NS
atenolol	267.170	3.44	5	10	SP
hydromorphone	286.144	3.55	5	5	SP
ranitidine	315.149	3.66	10	10	SP
phenylpropanolamine	152.107	3.83	25	25	OK
phenethylamine	122.096	4.23	5	5	BP
psilocin	205.134	4.45	NS	NS	NS
tizanidine	254.026	4.51	5	5	OK
dihydrocodeine	302.175	4.67	5	5	OK
norcodeine	286.144	4.69	5	5	BP
pseudo/ephedrine	166.123	4.70	5	5	BP
naloxone	328.154	4.89	5	5	BP
codeine	300.159	5.10	5	5	BP
olanzapine	313.148	5.13	100	NS	BP
clonidine	230.025	5.20	5	5	BP
pregabalin	160.133	5.25	NS	NS	NS
gabapentin	172.133	5.29	*Unk	*Unk	*Unk
methylone	208.099	5.63	5	5	BP
norpheniramine	227.154	5.63	5	5	BP
doxylamine	271.180	6.00	5	5	BP
amphetamine	136.112	6.05	25	25	BP
mescaline	212.128	6.12	5	5	BP
noroxycodone	302.139	6.43	5	5	BP

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
pheniramine	241.170	6.52	5	5	BP
oxycodone	316.154	6.79	5	5	BP
scopolamine	304.154	6.88	5	5	BP
MDA	180.102	7.13	5	5	BP
methamphetamine	150.128	7.35	5	5	BP
norhydrocodone	286.144	7.62	5	10	BP
O-desmethyltramadol	250.180	7.65	5	5	BP
6-AM	328.154	7.66	5	5	BP
PMA	166.123	7.94	5	5	BP
baclofen	214.063	8.04	NS	NS	I
hydrocodone	300.159	8.17	5	5	BP
MDMA	194.118	8.44	5	5	BP
OH-quetiapine	400.169	8.62	10	10	BP
phentermine	150.128	9.10	5	5	BP
quinine/quinidine	325.191	9.46	I	I	I
PMMA	180.138	9.54	5	5	BP
butylone	222.112	9.85	5	5	BP
tetrahydrozoline	201.139	9.87	5	5	BP
mephedrone	178.123	10.30	5	5	OK
lidocaine	235.180	10.37	5	5	OK
MDEA	208.133	10.72	5	5	OK
norketamine	224.084	10.83	5	5	OK
strychnine	335.175	10.84	5	5	OK
ropinirole	261.196	11.08	5	5	OK
atropine	290.175	11.16	5	5	OK
ketamine	238.099	11.16	5	5	OK
7-aminoclonazepam	286.074	11.19	5	5	OK
zolpidem metabolite	338.150	11.19	5	5	OK
mirtazapine	266.165	11.34	5	5	OK
norfentanyl	233.165	11.37	5	5	OK
desmethylvenlafaxine	264.196	11.38	5	5	OK
MBDB	208.133	11.50	5	5	OK
metoclopramide	300.147	11.60	5	5	OK
benzoylecgonine	290.139	11.70	5	5	OK
dextrorphan	258.185	11.84	5	5	OK
lamotrigine	256.015	11.89	5	5	OK
methoxetamine	248.165	11.92	5	5	OK

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
molindone	277.191	11.95	NS	NS	NS
tramadol	264.196	11.99	5	5	OK
metoprolol	268.194	12.00	5	5	OK
OH-bupropion	256.110	12.00	5	5	OK
pentylone	236.128	12.00	5	5	OK
zopiclone	389.112	12.00	10	5	OK
N-desmethyltramadol	250.180	12.08	5	5	OK
methylphenidate	234.149	12.10	5	5	OK
desloratadine	311.131	12.11	25	25	I
desmethyltapentadol	208.170	12.20	5	5	OK
alpha-PVP	232.170	12.29	5	5	OK
tapentadol	222.185	12.31	5	5	OK
desmethylclozapine	313.121	12.44	10	10	OK
7-aminoflunitrazepam	284.119	12.51	5	5	OK
chlorpheniramine	275.131	12.52	5	5	OK
MDPV	276.159	12.57	5	5	OK
carbinoxamine	291.126	12.62	5	5	OK
cocaine	304.154	12.62	5	5	OK
normeperidine	234.149	12.64	5	5	OK
risperidone	411.219	12.68	5	5	OK
meperidine	248.165	12.69	5	5	OK
OH-risperidone	427.214	12.71	5	5	OK
guaifenesin	199.096	12.75	NS	NS	NS
lacosamide	251.139	12.79	NS	NS	NS
zolpidem	308.176	12.79	5	5	OK
brompheniramine	319.080	12.83	5	5	OK
norchlordiazepoxide	286.074	12.88	5	5	OK
triprolidine	279.186	12.91	5	5	BP
clozapine	327.137	12.94	5	5	OK
LSD	324.207	12.97	5	5	SP
bupropion	240.115	12.98	5	5	OK
norbuprenorphine	414.264	12.99	5	5	OK
chlordiazepoxide	300.090	13.02	5	5	OK
venlafaxine	278.211	13.04	5	5	OK
norquetiapine	296.122	13.12	5	5	OK
acetylfentanyl	323.212	13.14	5	5	OK
TFMPP	231.110	13.15	5	5	OK

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
buspirone	386.255	13.23	5	5	OK
bupivacaine	289.227	13.28	5	5	I
trazodone	372.159	13.30	5	5	OK
cocaethylene	318.170	13.34	5	5	OK
methocarbamol	242.102	13.37	NS	NS	NS
phenycyclidine	244.206	13.48	5	5	OK
quetiapine	384.174	13.48	5	5	OK
alfentanyl	417.261	13.60	5	5	OK
propranolol	260.165	13.60	5	10	OK
donepezil	380.222	13.61	5	5	OK
dextromethorphan	272.200	13.70	5	5	OK
U47700	329.118	13.72	5	5	SP
OH-midazolam	342.080	13.76	5	5	I
mesoridazine	387.156	13.79	5	5	OK
3-methoxyPCP	274.217	13.80	5	5	OK
fentanyl	337.227	13.80	5	5	OK
midazolam	326.085	13.81	5	5	OK
ziprasidone	413.120	13.82	25	100	OK
medazepam	271.100	13.88	5	5	OK
flurazepam	388.159	13.94	5	5	OK
diphenhydramine	256.170	14.00	5	5	OK
furanylfentanyl	375.207	14.00	5	5	OK
desmethylcitalopram	311.155	14.04	5	5	OK
amoxapine	314.105	14.08	5	5	OK
25H-NBOME	302.175	14.10	5	5	OK
desmethyldoxepin	266.154	14.10	5	5	OK
buprenorphine	468.311	14.14	5	5	OK
citalopram	325.171	14.15	5	5	OK
carfentanil	395.233	14.19	5	5	OK
doxepin	280.170	14.21	5	5	OK
diltiazem	415.169	14.22	5	5	OK
butyrylfentanyl	351.243	14.27	5	5	OK
loxapine	328.121	14.31	5	10	I
brexpiprazole	434.190	14.32	5	10	OK
haloperidol	376.149	14.33	5	5	OK
asenapine	286.099	14.34	5	10	I, MI

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
iloperidone	427.203	14.36	5	5	OK
promethazine	285.142	14.40	5	5	OK
EDDP	278.190	14.46	5	5	OK
atomoxetine	256.170	14.50	5	5	OK
orphenadrine	270.185	14.50	5	5	OK
paroxetine	330.150	14.58	5	5	OK
25I-NBOH	414.056	14.61	5	5	OK
desipramine	267.186	14.64	5	5	OK
desmethylcyclobenzaprine	262.159	14.65	5	5	OK
perphenazine	404.156	14.66	NS	NS	NS
protriptyline	264.175	14.67	5	5	OK
bromazepam	316.008	14.70	5	10	OK
amlodipine	409.152	14.72	25	25	OK
cyproheptadine	288.175	14.74	5	5	OK
norverapamil	441.275	14.75	5	5	OK
imipramine	281.201	14.76	5	5	OK
norchlorcyclizine	287.131	14.76	25	25	I
cyclobenzaprine	276.175	14.77	5	5	OK
fluvoxamine	319.163	14.77	5	5	OK
hydroxyzine	375.183	14.77	5	5	OK
25C-NBOME	336.136	14.78	5	5	OK
norpropoxyphene	326.211	14.79	5	5	OK
nortriptyline	264.175	14.83	5	5	OK
aripiprazole	448.155	14.84	25	25	OK
duloxetine	298.126	14.84	5	10	OK
maprotyline	278.190	14.86	5	5	OK
verapamil	455.290	14.86	5	5	OK
fexofenadine	502.295	14.87	5	5	OK
MT45	349.264	14.90	5	5	BP
prochlorperazine	374.145	14.90	25	25	BP
25I-NBF	416.052	14.92	5	5	OK
propoxyphene	340.227	14.94	5	5	OK
25B-NBOME	380.086	14.95	5	5	OK
amitriptyline	278.190	14.95	5	5	OK
desmethyltrimipramine	281.201	14.97	5	5	OK
25I-NBMD	442.051	15.00	5	5	OK
methadone	310.217	15.02	5	5	OK

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
norfluoxetine	296.126	15.02	5	5	OK
benztropine	308.201	15.04	5	5	OK
trimipramine	295.217	15.08	5	5	OK
norsertraline	292.065	15.10	25	25	OK
25I-NBOME	428.072	15.15	5	5	OK
fluphenazine	438.182	15.15	5	10	OK
fluoxetine	310.141	15.18	5	5	OK
sertraline	306.081	15.24	5	5	OK
cetirizine	389.163	15.26	10	5	I
chlorpromazine	319.103	15.26	5	5	OK
desmethylclomipramine	301.147	15.32	5	5	OK
trifluprom azine	408.172	15.44	5	10	OK
clomipramine	315.162	15.45	5	5	OK
benzocaine	166.086	15.59	5	I	I
lurasidone	493.263	15.62	5	5	OK
loperamide	477.230	15.77	5	5	OK
thioridazine	371.161	15.80	100	100	OK
OH-alprazolam	325.085	15.86	5	5	OK
OH-triazolam	359.046	15.90	NS	NS	NS
loratadine	383.152	15.93	5	5	I
zaleplon	306.135	16.08	NS	NS	NS
nitrazepam	282.087	16.26	5	5	OK
estazolam	295.075	16.30	5	5	OK
desmethylflunitrazepam	300.078	16.34	NS	NS	NS
tetrazepam	289.111	16.37	5	5	OK
alprazolam	309.090	16.55	5	5	OK
flubromazolam	371.030	16.58	5	5	OK
lorazepam	321.019	16.58	NS	NS	NS
nordiazepam	271.063	16.60	5	5	OK
meclizine	391.194	16.63	5	5	OK
clonazepam	316.048	16.77	NS	NS	NS
triazolam	343.051	16.79	5	5	OK
metaxolone	222.112	16.81	NS	NS	NS
desalkylflurazepam	289.054	17.10	5	5	OK
etizolam	343.077	17.11	5	5	OK
flunitrazepam	314.094	17.35	NS	NS	NS
temazepam	301.074	17.40	NS	NS	NS

Analytes Sorted by Retention Time			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
lormetazepam	335.035	17.75	NS	NS	NS
phenazepam	348.972	17.81	100	NS	NS
nifedipine	347.124	18.13	NS	NS	NS
diazepam	285.079	18.14	5	5	OK
clobazam	301.075	18.36	NS	NS	NS
W18	422.094	20.04	NS	NS	NS
prazepam	325.111	20.23	5	5	OK
suvorexant	451.164	20.74	5	5	OK

Not suitable for analysis by this method	NS
LOD not thoroughly evaluated but consistently present in control spiked at 5 µg/mL	Unk*
Peak shape is broad; less than ideal	BP
Compound known to give split peaks	SP
Compound validated with no issues	OK
Matrix Interference	I